

## Comparative Evaluation of Biomed InTray® Colorex MRSA with BD ESwab Collection Kit/ BBL™ CHROMagar® MRSA II

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### Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most dangerous antibiotic resistant pathogens and a common cause of most health-care acquired infections. MRSA causes skin, wound and bloodstream infections that can cause sepsis and ultimately lead to death. CDC and WHO have listed MRSA as a serious threat infection and included in The National Action Plan for Combating Antibiotic-resistant Bacteria. Early, reliable, and accurate diagnosis of MRSA in a clinical setting is critical for the treatment and control of infection in hospitals and the community. We comparatively evaluated the efficacy of two commercial diagnostic systems, Biomed InTray® Colorex and BDTM ESwab Regular Collection Kit/ BBL™ CHROMagar® (ESwab + CHROMagar®) to recover 51 MRSA clinical isolates. The percentage recovery of MRSA clinical isolates in InTray® and in ESwab + CHROMagar® was 99% and 75%, respectively. Our findings suggest that InTray® was more efficient than ESwab + CHROMagar® in recovering MRSA clinical isolates.

**Keywords:** Methicillin-resistant *S. aureus* (MRSA); Diagnostic test; InTray®; ESwab+CHROMagar®

### Introduction

Antimicrobial resistance has developed over time, usually through evolution of the bacteria, however it has been further accelerated because of misuse and overuse of antibiotics. As a result, the antimicrobial drug becomes ineffective in killing the bacteria, resulting in persistent infections, prolonged illness and ultimately increasing the cost of health care across the globe. Such bacteria are also referred to as “Superbugs” because infections caused by them are extremely difficult to treat [1]. Many strains of *S. aureus* have developed resistance to methicillin, oxacillin and nearly all the beta-lactam antibiotics by producing an alternative penicillin-binding protein known as PBP2a that are collectively referred to as “Methicillin-resistant *Staphylococcus aureus*” or “MRSA” [2]. PBP2a is encoded by the *mecA* gene and has a low affinity to many beta-lactam antibiotics [3].

MRSA is one of the most dangerous antibiotic-resistant pathogens and one of the leading causes of hospital acquired infections (HAIs), in addition, MRSA is rapidly spreading within the community. The typical sites of colonization of the bacteria are the nostrils and mucous membranes of the upper respiratory tract. It can cause lethal infections such as infective endocarditis (IE), skin and soft tissue infection (SSTI), and hospital-acquired/ventilator acquired pneumonia (HAP) [4,5]. The Center for Disease Control (CDC) and the World Health Organization (WHO) have listed MRSA as a “serious threat” and it is on high priority list on health challenges faced globally by practitioners. In the United States, the National Health Care Safety Network estimates that hospitalized patients acquire 2 million HAIs per year, out of which a large percentage are related to MRSA infections [6]. The costs are estimated in the billions of dollars, making the economic burden of disease very high. According to the CDC, MRSA leads to 10,000 deaths in the U.S. per year [6].

To provide effective and targeted treatment for MRSA to the patient, the first step is identifying the infection as MRSA from the multitude of infectious bacterium. Screening usually involves swabbing of the nasal passages in a conventional selective media (Amies media) and then transporting to the clinical laboratory for bacterial culture. Molecular diagnostic methods such as PCR [7], which may provide rapid detection, have been cited with reports of false positive results

where patients have been diagnosed as MRSA positive [8]. Other concerns regarding this method include the associated costs of trained technicians, specialized machinery, and consumables [9]. The ease at which conventional diagnosis methods are performed in laboratories with limited resources continues to underscore the importance of non-molecular diagnostic testing as reliable and cost-efficient. Thus, we comparatively evaluated two commercially available non-molecular diagnostics systems, BioMed InTray® Colorex (InTray®) and the conventional BDTM ESwab Collection Kit/ BBL™ CHROMagar® (ESwab + CHROMagar®).

### Materials and Methods

#### Test articles

InTray® Colorex MRSA (InTray®) Lot # 6VA139X; Expiration: 11-12-2016) was provided by Biomed Diagnostics, Inc. Conventional products, BDTM ESwab collection kit (ESwab) [Lot # 1MMJ45; Expiration: 05-31-2017) and BBL™ CHROMagar® MRSA II (CHROMagar) (Lot # 6244574; Expiration: 11-18-2016) were purchased from Becton, Dickinson and Company.

#### Bacterial strains

This study was carried out to compare the recovery of MRSA clinical isolates on the InTray® and ESwab + CHROMagar® diagnostic systems. All the clinical isolates acquired from Eurofin Medinet, Inc. (Herndon, VA) were Oxacillin resistant and 100% susceptible to Vancomycin, Daptomycin, and Linezolid. ATCC 29213 which is a Wild

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type QC susceptible strain of *S. aureus* was used as a negative control. ATCC 29213 was obtained from the American Type Culture Collection (Manassas, VA). The strains were maintained as frozen glycerol stocks at -80°C. Working stocks were prepared by thawing a glycerol stock, streaking onto a Tryptic Soy Agar plate and incubated at 37°C for 24 hours.

## Methods

Antimicrobial susceptibility testing was performed on 51 clinical isolates as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Overnight cultures for each of the isolates were used to dilute stock solutions of microorganism's equivalent to a 0.5 McFarland standard. These were prepared in cation-adjusted Mueller-Hinton broth (CAMHB) to achieve an inoculum of  $1.5 \times 10^8$  CFU/mL [10]. This inoculum was diluted further in CAMHB to achieve  $3 \times 10^3$  CFU/mL for each strain and 50 µL of this inoculum containing 150 CFU was plated on the InTray® and the ESwab + CHROMagar®. The test was performed in triplicates. To determine the bacterial recovery in the InTray™, 50 µL of inoculum containing 150 CFU was plated,

incubated at room temperature for 48 hours and then incubated at 37°C for additional 24 hours. This 48 hour incubation at room temperature was done to simulate shipping conditions while samples are in transit before they reach the clinical lab testing facilities. To test the ESwab + CHROMagar®, 1 mL of Amies media was inoculated with 50 µL of inoculum containing 150 CFU. The inoculum was incubated at room temperature for 48 hours, then plated on CHROMagar® plates, and incubated at 37°C for additional 24 hours. After the incubation, the recovery efficacy was determined by enumerating the number of colonies in the InTray® and CHROMagar® plates. Growth of pink-pigmented colonies was considered as positive (indicating MRSA) and no growth of colonies, or with other colors, was considered as negative.

## Results

The 51 clinical isolates were categorized based on the susceptibility or resistance to the following 5 antibiotics: Ciprofloxacin, Clindamycin, Erythromycin, Imipenem and Sulfamethoxazole/Trimethoprim. The antibiogram shown in Table 1 confirms that MRSA isolates are multi-drug resistant. In addition, clinically important strains ATCC 33591

Serial #	Organism	Isolate #	Antibiotic Resistance	Ciprofloxacin		Clindamycin		Erythromycin		Imipenem		Trimethoprim/ Sulfamethoxazole	
			No. of Antibiotics that are Ineffective	MIC (µg/mL)	CLSI interpretation	MIC (µg/mL)	CLSI interpretation	MIC (µg/mL)	CLSI interpretation	MIC (µg/mL)	CLSI interpretation	MIC (µg/mL)	CLSI interpretation
1	<i>Staphylococcus aureus</i>	1974126	4	>16	R	>4	R	>8	R	>32	R	≤ 0.25	S
2	<i>Staphylococcus aureus</i>	1974131	4	>16	R	>4	R	>8	R	16	R	≤ 0.25	S
3	<i>Staphylococcus aureus</i>	1974129	3	16	R	>4	R	>8	R	0.5	S	≤ 0.25	S
4	<i>Staphylococcus aureus</i>	1974133	3	16	R	>4	R	>8	R	2	S	≤ 0.25	S
5	<i>Staphylococcus aureus</i>	1974136	3	>16	R	>4	R	>8	R	0.5	S	≤ 0.25	S
6	<i>Staphylococcus aureus</i>	1974140	3	>16	R	>4	R	>8	R	0.5	S	≤ 0.25	S
7	<i>Staphylococcus aureus</i>	1974143	2	16	R	0.12	S	>8	R	0.25	S	≤ 0.25	S
8	<i>Staphylococcus aureus</i>	1974119	2	>16	R	0.12	S	>8	R	0.25	S	≤ 0.25	S
9	<i>Staphylococcus aureus</i>	1974120	2	>16	R	0.12	S	>8	R	0.25	S	≤ 0.25	S
10	<i>Staphylococcus aureus</i>	1974121	4	>16	R	>4	R	>8	R	32	R	≤ 0.25	S
11	<i>Staphylococcus aureus</i>	1974135	4	>16	R	>4	R	>8	R	32	R	≤ 0.25	S
12	<i>Staphylococcus aureus</i>	1974148	3	>16	R	0.25	S	>8	R	16	R	≤ 0.25	S
13	<i>Staphylococcus aureus</i>	1974155	3	16	R	>4	R	>8	R	2	S	≤ 0.25	S
14	<i>Staphylococcus aureus</i>	1974161	3	>16	R	>4	R	>8	R	0.25	S	≤ 0.25	S
15	<i>Staphylococcus aureus</i>	1974171	3	>16	R	>4	R	>8	R	0.125	S	≤ 0.25	S
16	<i>Staphylococcus aureus</i>	1974175	2	16	R	0.12	S	>8	R	1	S	≤ 0.25	S
17	<i>Staphylococcus aureus</i>	1974122	2	>16	R	0.12	S	>8	R	0.12	S	≤ 0.25	S
18	<i>Staphylococcus aureus</i>	1974123	2	0.5	S	>4	R	>8	R	2	S	≤ 0.25	S
19	<i>Staphylococcus aureus</i>	1974125	2	16	R	0.12	S	>8	R	0.25	S	≤ 0.25	S
20	<i>Staphylococcus aureus</i>	1974127	2	>16	R	0.12	S	>8	R	0.5	S	≤ 0.25	S

21	<i>Staphylococcus aureus</i>	1974132	2	>16	R	0.12	S	>8	R	0.06	S	≤ 0.25	S
22	<i>Staphylococcus aureus</i>	1974137	2	4	R	0.12	S	>8	R	0.06	S	≤ 0.25	S
23	<i>Staphylococcus aureus</i>	1974138	2	16	R	0.12	S	>8	R	0.12	S	≤ 0.25	S
24	<i>Staphylococcus aureus</i>	1974139	2	>16	R	0.12	S	>8	R	0.5	S	≤ 0.25	S
25	<i>Staphylococcus aureus</i>	1974144	2	16	R	0.12	S	>8	R	0.12	S	≤ 0.25	S
26	<i>Staphylococcus aureus</i>	1974145	2	>16	R	0.25	S	>8	R	0.25	S	≤ 0.25	S
27	<i>Staphylococcus aureus</i>	1974152	2	16	R	0.12	S	>8	R	0.5	S	≤ 0.25	S
28	<i>Staphylococcus aureus</i>	1974153	2	8	R	0.06	S	>8	R	8	I	≤ 0.25	S
29	<i>Staphylococcus aureus</i>	1974156	1	0.5	S	0.12	S	>8	R	0.5	S	≤ 0.25	S
30	<i>Staphylococcus aureus</i>	1974128	1	0.5	S	0.12	S	>8	R	0.5	S	≤ 0.25	S
31	<i>Staphylococcus aureus</i>	1974141	1	0.5	S	0.12	S	>8	R	0.12	S	≤ 0.25	S
32	<i>Staphylococcus aureus</i>	1974142	1	0.5	S	0.12	S	>8	R	0.12	S	≤ 0.25	S
33	<i>Staphylococcus aureus</i>	1974146	1	0.5	S	0.12	S	>8	R	0.12	S	≤ 0.25	S
34	<i>Staphylococcus aureus</i>	1974147	1	0.5	S	0.12	S	>8	R	0.5	S	≤ 0.25	S
35	<i>Staphylococcus aureus</i>	1974150	1	16	R	0.12	S	0.25	S	0.06	S	≤ 0.25	S
36	<i>Staphylococcus aureus</i>	1974162	1	>16	R	0.12	S	0.5	S	≤ 0.06	S	≤ 0.25	S
37	<i>Staphylococcus aureus</i>	1974169	1	>16	R	0.12	S	0.5	S	≤ 0.06	S	≤ 0.25	S
38	<i>Staphylococcus aureus</i>	1974170	1	>16	R	0.12	S	0.5	S	≤ 0.06	S	≤ 0.25	S
39	<i>Staphylococcus aureus</i>	1974176	1	>16	R	0.12	S	0.5	S	0.5	S	≤ 0.25	S
40	<i>Staphylococcus aureus</i>	1974177	1	>64	R	0.12	S	>4	R	≤ 0.06	S	≤ 0.25	S
41	<i>Staphylococcus aureus</i>	1974184	1	0.125	S	0.12	S	>4	R	≤ 0.06	S	≤ 0.25	S
42	<i>Staphylococcus aureus</i>	1974199	1	>64	R	0.12	S	>4	R	≤ 0.06	S	≤ 0.25	S
43	<i>Staphylococcus aureus</i>	1974201	1	4	R	0.12	S	>4	R	≤ 0.06	S	≤ 0.25	S
44	<i>Staphylococcus aureus</i>	1974202	1	0.5	S	0.12	S	0.5	S	1	S	≤ 0.25	S
45	<i>Staphylococcus aureus</i>	1974130	0	0.25	S	0.12	S	0.25	S	0.12	S	≤ 0.25	S
46	<i>Staphylococcus aureus</i>	1974134	0	0.12	S	0.12	S	0.5	S	0.25	S	≤ 0.25	S
47	<i>Staphylococcus aureus</i>	1974149	0	0.12	S	0.12	S	0.5	S	0.25	S	≤ 0.25	S
48	<i>Staphylococcus aureus</i>	ATCC 33591	1	0.125	S	>64	R	>64	R	1	S	1	S
49	<i>Staphylococcus aureus</i>	USA300	1	0.125	S	0.125	S	32	R	≤ 0.06	S	0.25	S
50	<i>Staphylococcus aureus</i>	1974151	0	0.5	S	0.12	S	0.5	S	0.12	S	≤ 0.25	S
51	<i>Staphylococcus aureus</i>	1974158	0	0.5	S	0.12	S	0.5	S	0.5	S	≤ 0.25	S

Clinical Isolates obtained from Eurofins Medinet, Inc. Herndon, VA except USA 300 and ATCC 33591 were obtained from ATCC. NOTE: All isolates listed were oxacillin resistant and 100% susceptible to vancomycin, daptomycin, and linezolid.

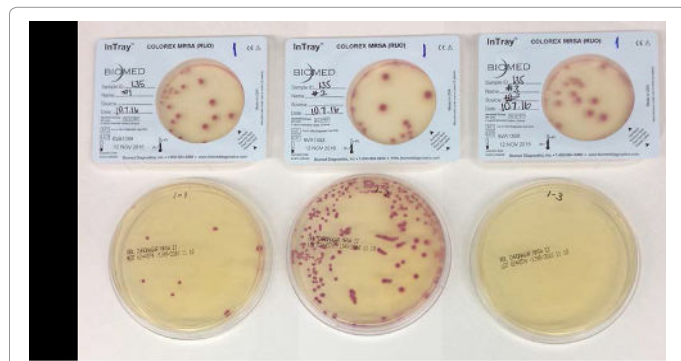
**Table 1:** Antibiogram of methicillin resistant *S. aureus* (MRSA) clinical isolates used in the current study. This confirms that MRSA isolates used in the study are indeed multi-drug resistant.

Serial #	Isolate #	# of Antibiotic Resistant	In Tray				CHROMagar				
			1	2	3	Average	1	2	3	Average	
1	1974126	4	51	TNTC	TNTC	TNTC	TNTC	250	89	TNTC	
2	1974131	4	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	
3	1974129	3	57	42	51	50	0	0	0	0	
4	1974133	3	43	50	54	49	44	62	TNTC	TNTC	
5	1974136	3	49	54	50	51	1	0	0	1	
6	1974140	3	0	0	0	0	0	0	0	0	
7	1974143	3	TNTC	TNTC	TNTC	TNTC	0	0	0	0	
8	1974119	2	TNTC	37	56	TNTC	0	0	0	0	
9	1974120	2	26	33	31	30	0	0	0	0	
10	1974121	2	46	18	41	35	TNTC	10	TNTC	TNTC	
11	1974135	4	42	36	23	34	13	TNTC	0	TNTC	
12	1974148	4	42	57	40	46	TNTC	TNTC	TNTC	TNTC	
13	1974155	3	76	61	48	662	TNTC	TNTC	TNTC	TNTC	
14	1974161	3	12	17	27	19	TNTC	TNTC	TNTC	TNTC	
15	1974171	3	47	46	50	48	TNTC	TNTC	TNTC	TNTC	
16	1974175	3	43	46	50	46	TNTC	TNTC	TNTC	TNTC	
17	1974122	2	38	40	54	44	0	0	0	0	
18	1974123	2	56	46	55	52	0	0	0	0	
19	1974125	2	39	40	47	42	0	0	0	0	
20	1974127	2	55	55	127	79	0	0	0	0	
21	1974132	2	30	26	60	39	0	48	29	26	
22	1974137	2	34	45	58	46	1	0	1	1	
23	1974138	2	35	41	25	34	TNTC	TNTC	TNTC	TNTC	
24	1974139	2	57	53	67	59	0	0	0	0	
25	1974144	2	46	56	47	50	TNTC	TNTC	TNTC	TNTC	
26	1974145	2	46	35	60	47	0	0	0	0	
27	1974152	2	34	33	36	34	TNTC	TNTC	TNTC	TNTC	
28	1974153	2	28	51	57	45	0	0	0	0	
29	1974156	2	8	1	2	4	TNTC	TNTC	TNTC	TNTC	
30	1974128	1	27	31	28	29		1	0	0	
31	1974141	1	51	36	60	49	0	11	0	4	
32	1974142	1	55	74	73	67	2	1	0	1	
33	1974146	1	68	78	40	62	0	0	0	1	
34	1974147	1	55	57	39	50	2	2	2	2	
35	1974150	1	85	75	98	86	3	TNTC	TNTC	TNTC	
36	1974162	1	34	53	42	43	TNTC	2	1	1	
37	1974169	1	61	38	56	52	0	TNTC	TNTC	TNTC	
38	1974170	1	43	56	43	47	TNTC	TNTC	TNTC	TNTC	
39	1974176	1	26	34	32	31	TNTC	TNTC	TNTC	TNTC	
40	1974177	1	40	46	56	47	TNTC	TNTC	TNTC	TNTC	
41	1974184	1	43	38	34	40	TNTC	TNTC	TNTC	TNTC	
42	1974199	1	34	45	38	39	TNTC	TNTC	TNTC	TNTC	
43	1974201	1	34	36	51	40	TNTC	TNTC	TNTC	TNTC	
44	1974202	1	47	42	41	43	TNTC	TNTC	TNTC	TNTC	
45	1974130	0	48	39	44	44	1	1	1	1	
46	1974134	0	28	40	61	43	0	0	0	0	
47	1974149	0	67	43	64	58	0	6	11	6	
48	ATCC33591	1	30	35	50	38	TNTC	TNTC	TNTC	TNTC	
49	USA 300	1	37	26	29	31	0	1	74	25	
50	1974151	0	83	59	56	66	5	6	5	5	
51	1974158	0	64	62	49	58	TNTC	TNTC	TNTC	TNTC	
Total Number of Strains Recovered						In Tray					CHROMagar
						50/51					38/51
Percentage of Recovery						99%					75%
Positive for bacterial growth						Negative for bacterial growth					
TNTC	Too Numerous to count >300 colonies										

Table 2: Recovery data for 51 MRSA clinical isolates in InTray® and ESwab + CHROMagar®.



**Figure 1:** Representative result showing good recovery of MRSA strain (Eurofin isolate # 1974134) in InTray® but not in ESwab + CHROMagar®. The study was carried out in triplicates. Pink colonies represent positive for MRSA growth.



**Figure 2:** Representative result where the MRSA strain (Eurofin isolate # 1974135) was recovered within all the triplicates in InTray®, whereas one of the triplicates in ESwab + CHROMagar® did not recover the bacteria. The study was carried out in triplicates. Pink colonies represent positive for MRSA growth.

and ATCC BAA- 1717 USA 300 were included to further validate the study.

We evaluated the recovery of 51 MRSA strains in InTray® and ESwab + CHROMagar® diagnostic systems. Out of the 51 MRSA clinical isolates tested, 50 MRSA isolates were recovered in InTray® whereas only 38 strains were recovered in ESwab + CHROMagar® (Table 2). Figure 1 is a representative picture showing recovery of MRSA strain (Eurofin isolate # 1974134) in InTray® but not in ESwab + CHROMagar®. Recovery of MRSA strains were consistent within the triplicates in InTray®. In contrast, there was variation in recovery within the triplicates in ESwab + CHROMagar®. Figure 2 is a representative result where the MRSA strain (Eurofin isolate # 1974135) was recovered within all the InTray® triplicates whereas one of the triplicates in ESwab + CHROMagar® did not recover the bacteria. One MRSA strain (Eurofin isolate # 1974140) was not recovered in InTray® or in ESwab + CHROMagar®. ATCC 29213 which is a Wild type QC susceptible strain of *S. aureus* was used as a negative control and it did not grow on both the InTray® and CHROMagar®, confirming that both the systems are selective to MRSA isolates.

## Discussion

The impact of MRSA on hospitals and the community remains a burden in both developed and developing countries. Recent data reported to the National Healthcare Safety Network (NHSN) on MRSA bloodstream infections in the United States, indicates that by the end

of 2015 there was little change in the average facility Standardized Infection Ratio (0.988), compared to a 2010-2011 baseline and is significantly increased compared to the previous year statistics [6,11]. Hence, an effective, easy to use and simple diagnostic system is much needed.

The current study was carried out to compare and evaluate the recovery efficacy of Biomed InTray® Colorex MRSA with a conventional product, BDTM ESwab collection kit and BBL™ CHROMagar® MRSA II. The current study evaluated the limit of detection in both the diagnostic systems by using a low inoculum size and determine if these diagnostic systems were effective in recovering the MRSA isolates.

The conventional method for MRSA diagnosis usually involves swabbing the patient sample using ESwab, which is then incubated with Amies media and then transported to a clinical laboratory, where the patient sample is then streaked for isolation on a selective media such as CHROMagar® [6,12,13]. In developing countries, the clinical laboratory may not be accessible and hence the transit time may take 1-2 days before the patient's sample can be cultured and streaked to a selective agar for diagnosis. This may lead to significant loss of viability of the bacteria present in the patient sample. There have been few studies that have measured bacterial cell viability in Amies media, e.g. Robinson et al. demonstrated that 10 MRSA isolates used in their study could survive in Amies media at room temperature up to 14 days [14]. However, our results demonstrate that not all MRSA clinical isolates used in this study could be recovered on ESwab + CHROMagar® suggesting potential loss of viability of low bacterial load in transportation media. The percentage recovery of MRSA isolates in ESwab + CHROMagar® was only 75%. In contrast, InTray® demonstrated a higher percentage recovery of 99%.

Additionally, performing the testing with InTray® was easier and faster than ESwab + CHROMagar® which makes the InTray® system practically more “user friendly” with less room for error and contamination. The InTray® system has significant benefits over the ESwab + CHROMagar®. The InTray® system consists of a re-closable outer seal containing an optically clear, anti-fog window, which creates an airtight 2” diameter chamber providing a large enough area to streak for isolation. It is convenient to use as it combines collection, culture, and observation into one device. Minimal laboratory procedures and equipment are needed, and the device is easier to store. Because it is fully enclosed, the InTray® system prevents contamination and it also easy to see presence or absence of growth. There have been reports that newer molecular diagnostics such as rapid PCR testing have some issues with false-positive results, in which a high proportion (12.9%) of the patients were wrongly determined to be MRSA positive patients [8]. Hence, there is a need for a reliable diagnostic tool, and our data suggest that the InTray® system offers a robust, non-molecular solution for screening MRSA.

## Conclusion

This study demonstrates that, in comparison with ESwab + CHROMagar®, the InTray® diagnostic system efficiently recovered more MRSA clinical isolates. The percentage of MRSA strains recovered in InTray® was 99% and in ESwab + CHROMagar® was 75%, suggesting InTray® may provide improved efficiency, however, additional studies are needed to confirm these findings in a clinical setting.

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